Against the backdrop of less fossil fuel and more severe environmental pollution, energy recovery from organic residues is becoming a more attractive proposition. Biogas, produced by microorganisms during anaerobic biomass fermentation, consists primarily of CH₄ (40-75%) and CO₂ (15-60%), as well as H₂S (0.005-2%) and other trace components [1]. After desulphurization and dehydration, biogas can be used to generate heat and electricity. After further upgrading process to increase the concentration...
of CH₄ and reduce impurities, biogas can be transformed into biomethane and applied as a substitute of natural gas.

The H₂S in biogas is mainly related to the anaerobic degradation of S-containing organic material such as sulfolipid or amino acid, or formatted by sulfate reduction, where sulfate is used as the terminal electron acceptor [2]. The H₂S content depends on the type of organic substrates for fermentation. The fermentation of manure or food waste shows typical H₂S concentrations in the range of 2,000-6,000 ppmv in biogas, while for anaerobic wastewater treatment in the paper industry, H₂S concentration can be measured at up to 30,000 ppmv [3].

H₂S can cause corrosion in pipelines and equipment, along with high toxicity for health and the environment. Therefore, it is usually removed in an early state of the biogas upgrading process. A variety of methods have been used for desulphurization, which can be classified as physical, chemical, and biological methods according to principle, or as in-situ and external according to process, or as rough and fine desulphurization according to purification level. The comparative overview of these methods is given elsewhere [1, 4-5]. The method or combination of methods for desulphurization can be determined based on the biogas composition and subsequent utilization.

Dosing iron compounds (especially iron salts) directly into the fermenter is an in-situ method for rough desulphurization. This desulphurization method has the advantages of simple operation, small investment, and good desulphurization rate. Five kinds of natural iron ores were used as in-situ desulphurizers during the anaerobic digestion of waste-activated sludge, and limonite showed high desulphurization efficiency [6]. Besides being used as H₂S control in anaerobic fermenters, iron compounds have been widely used for the abatementsulfide-associated problems in sewer systems [7-8]. The reactions among iron and sulfide species in these aqueous phases are complex and have not yet been unequivocally ascertained and quantified [9].

Under the most common description, the main desulphurization interactions occurring in anaerobic fermenters are shown in Equations 1 and 2 [1, 9]. Fe (II) can remove sulfide by forming ferrous sulfide precipitation. Fe (III) can remove sulfide by oxidizing it to sulfur while being reduced to Fe (II), which can subsequently produce ferrous sulfide.

\[
\begin{align*}
\text{Fe}^{2+} + \text{HS}^- & \rightarrow \text{FeS} + \text{H}^+ \\
2\text{Fe}^{3+} + \text{HS}^- & \rightarrow 2\text{Fe}^{2+} + \text{S} + \text{H}^+ 
\end{align*}
\]  

In this study, different iron compounds, including FeCl₂, FeCl₃, Fe(OH)₃, Fe₂O₃, and FeSO₄, were applied as in-situ desulphurizers in chicken manure (CM) fermentation to reduce the emission of H₂S. The biogas yield, CH₄ concentration, and H₂S concentration were examined to evaluate the performance of these desulphurizers. In order to establish the prediction model of the required amount for in-situ desulphurizer, it is assumed that the dosage of a desulphurizer could simply be divided into two parts, one part for consumption of released H₂S and the other part for guaranteeing a certain desulphurizing level. Under this assumption, the prediction formulas were fitted and applied successfully in a larger fermentation system.

Materials and Methods

Substrates and Inoculum

Two batches of fresh CM were successively collected from a chicken farm (DQY Ecological Farm, Beijing, China), labeled CM1 and CM2, respectively. The total solids (TS) and volatile solids (VS) were determined to be 29.1% (based on fresh mass) and 68.0% (based on TS) for CM1, and 30.7% and 35.1% for CM2. The digested effluent of the biogas plant feeding CM on the farm was used as inoculum. The TS of the inoculum was below 1%, so the contribution of inoculum to solid content was ignored in calculation.

Fermentation and in-situ Desulphurization

The batch fermentation of CM and in-situ desulphurization were taken in two kinds of apparatuses: 50 mL bottles and 5 L fermenters (BIOTECH-5JG-2, Baoxing Bio-Engineering Equipment Company, Shanghai, China). For all fermentation, the inoculum took 35% of the loading volume, and the initial TS content was adjusted to 7.0% through mixing CM, inoculum, and water. The bottles were loaded with 25 mL feed mixture and incubated at 37°C and 130 r min⁻¹. Five kinds of iron compounds (FeCl₂, FeCl₃, Fe(OH)₃, Fe₂O₃, and FeSO₄) were added into bottles as in-situ desulphurizers with the feedstock, respectively. For each iron compound, different initial concentrations based on the fermentation volume were applied, which were 0 (as a control), 2, 4, 8, 12, 16, and 32 mmol L⁻¹, respectively. In 5 L fermenters, the feeding volume was 3.5 L. The temperature was kept at 37°C and the stirring rate was 100 r min⁻¹. CM1 was the feedstock in bottles except that when Fe(OH)₃ was added as the desulphurizer, while CM2 was fed in 5 L fermenters as well as bottles adding Fe(OH)₃.

Prediction Model of Desulphurizer Dosage

The dosage of desulphurizers was assumed to be divided into two parts: one for consumption of released H₂S and the other for guaranteeing a certain desulphurizing level. It was calculated by Equation 3:
\[ m = m_r + xV \]  

(3)

…where \( m \) is the dosage of desulfurizer; \( m_r \) represents the amount of desulfurizer that reacted with reduced \( \text{H}_2\text{S} \), whereas the reduced \( \text{H}_2\text{S} \) can be calculated by subtracting the expected value of \( \text{H}_2\text{S} \) from the \( \text{H}_2\text{S} \) yield without desulfurizer added; \( x \) represents the needed concentration of desulfurizer maintained in the liquid for achieving a specific \( \text{H}_2\text{S} \) value in biogas; and \( V \) is the fermentation volume. The relationship between \( \text{H}_2\text{S} \) concentration in biogas and desulfurizer concentration in liquid (\( x \)) can be obtained by fitting experimental data during the middle period of the 50 mL fermentation.

**Analytical Methods**

TS and VS were determined according to the standard methods [10]. For 50 mL bottles, pH was measured using a pH meter (PHSJ-4A, REX Instrument Company, Shanghai, China), and biogas yield was determined by 100 mL syringe. For 5 L fermenters, pH was recorded automatically, and biogas yield was determined by the gas-collecting method of draining saturated \( \text{NaHCO}_3 \). The concentration of \( \text{CH}_4 \) was analyzed by gas chromatography (GC) with a thermal conductivity detector (TCD) (Model GC-2000III, Shanghai Institute of Computing Technology, China) and a packed TDX-01 column using \( \text{H}_2 \) as the carrier gas. The temperatures of the injector, column, and TCD were 150, 120, and 250ºC, respectively. The concentration of \( \text{H}_2\text{S} \) was analyzed by GC (Agilent 7890A, Agilent Technologies, USA) with a sulfur chemiluminescence detector (SCD) using a capillary column GS-GASPRO (60 m x 0.32 mm), and the carrier gas was He. The initial temperature of the column was 60ºC for three minutes. Then the column was heated to 200ºC at a rate of 10ºC min\(^{-1}\), and finally kept for 15 minutes. The temperatures of the injector, SCD, and burner were 250, 250, and 800ºC, respectively.

**Results and Discussion**

Selection of in-situ Desulfurizers

Five iron compounds (\( \text{FeCl}_2 \), \( \text{FeCl}_3 \), \( \text{Fe(OH)}_3 \), \( \text{Fe}_2\text{O}_3 \), and \( \text{FeSO}_4 \)) were applied as in-situ desulfurizers with different concentrations in 50 mL bottles. The characteristics of biogas production were shown in Fig. 1. Compared with the controls, the biogas yield and \( \text{CH}_4 \) concentration of the CM fermentation with desulfurizers showed no obvious differences, indicating that adding iron compounds did not cause significant inhibition or promotion to the biogas production. Iron is an essential trace element that is required by methanogens and other microorganisms during fermentation for electron transport and function of certain enzymes [11]. The optimum iron concentration was reported to range from 0.28 to 50.4 g m\(^{-3}\) [12]. Some studies have proven that adding iron could provide more biogas production and \( \text{CH}_4 \) content, especially in the mono-fermentation of agricultural crops, which suffers from a lack of trace elements easily [13-14]. But there are seldom reports about the deficiency of trace elements in CM fermentation, and in fact feeding with animal excrement can generally satisfy the demand for micronutrients [11]. Therefore, adding iron compounds had no significant influence on biogas production in this study. Zhou et al. found that adding limonite had different impacts on biogas production with different initial concentrations of sulfate, which might be due to the changes of microbial quantity and activity under different conditions [6].

The desulfurization rates of different desulfurizers at different concentrations are listed in Table 1. Combined with the \( \text{H}_2\text{S} \) concentration changes in Fig. 1, it clear that the \( \text{H}_2\text{S} \) content decreased obviously after adding desulfurizers. For each kind of iron compound, the more amounts added, the less \( \text{H}_2\text{S} \) was obtained. Among these five in-situ desulfurizers, \( \text{FeCl}_2 \), \( \text{FeCl}_3 \), and \( \text{Fe(OH)}_3 \) showed better performance; the desulfurization rates were all above 98.5% when the addition was 16 mmol L\(^{-1}\). Considering avoiding the introduction of other potentially polluting ions, \( \text{Fe(OH)}_3 \) was more environmentally friendly. Compared with the three desulfurizers mentioned above, the desulfurization efficiency of \( \text{Fe}_2\text{O}_3 \) was much lower, and when the addition was 16 mmol L\(^{-1}\), the desulfurization rate was only 90.5%. Due to its insolubility in water, \( \text{Fe}_2\text{O}_3 \) could not fully contact and react with \( \text{H}_2\text{S} \). So it is not a good choice as an in-situ desulfurizer. When \( \text{FeSO}_4 \) was applied, the desulfurization effect seemed normal in the early stage of fermentation, but later the \( \text{H}_2\text{S} \) concentration increased sharply – far higher than that of control (up to 9,000 ppmv). This indicated that the added \( \text{SO}_4^{2-} \) got involved in the microbial reaction process, employed by sulfate-reducing bacteria as an electron acceptor to generate \( \text{H}_2\text{S} \) [6, 15]. Therefore, \( \text{FeSO}_4 \) was not suitable for in-situ desulfurization use. In the following model calculation and experiments, the three desulfurizers with good performance (\( \text{FeCl}_2 \), \( \text{FeCl}_3 \), and \( \text{Fe(OH)}_3 \)) were applied.

**Prediction Model of Desulfurizer Dosage**

To determine the dosage of desulfurizer is key for the in-situ desulfurization process. But until now, there has been no public report about how to determine the desulfurizer addition. We supposed that the \( \text{H}_2\text{S} \) concentration was associated with the concentration of the desulfurizer in fermentation liquid. Through the experiments in 50 mL bottles, the fitted curve between the desulfurizer concentration in liquid and the \( \text{H}_2\text{S} \) concentration in biogas was acquired (Fig. 2). The fitting formula was shown as Equation 4 and the values of \( R^2 \) were all above 0.999.

\[
y = (a + bx)^{-1}
\]  

(4)

where \( x \) represents the desulfurizer content in the liquid and \( y \) represents the \( \text{H}_2\text{S} \) content in biogas. For \( \text{FeCl}_2 \), the values of \( a \), \( b \), and \( c \) were \(-5.09\times10^{-4} \), 0.0016, and
Fig. 1. Biogas yield, CH$_4$ concentration, and H$_2$S concentration when using a) FeCl$_2$, b) FeCl$_3$, c) Fe(OH)$_3$, d) Fe$_2$O$_3$, and e) FeSO$_4$ as in-situ desulfurizers with different concentrations in 50 mL bottles.
Selection of in-situ Desulfurizers...

1.171, respectively; for FeCl₃ the values of \(a\), \(b\), and \(c\) were \(2.295 \times 10^{-4}\), \(1.504 \times 10^{-4}\), and \(2.561\), respectively; for Fe(OH)₃, the values of \(a\), \(b\), and \(c\) were \(0.1572\), \(-0.1777\), and \(-0.2731\), respectively. In fact, the form of Equation 4 is not unchangeable and could be replaced by other forms, as long as it reflects the relationship between \(x\) and \(y\) in the concentration range of desulfurizer.

For example, when FeCl₂ was used as the in-situ desulfurizer, if the required H₂S content in biogas was 200-300 ppmv, according to Equation 4, the FeCl₂ concentration in liquid should maintain around 2.11-2.87 mmol L⁻¹; if the required H₂S content in biogas was 50 ppmv, the FeCl₂ concentration in liquid should be 8.83 mmol L⁻¹. It also can be seen from Fig. 2 that with the increase of desulfurization level, much more addition of desulfurizer would be needed, and the H₂S content in the biogas could not be reduced unboundedly.

In practical application, it is necessary to consider the trade-off between desulfurization level and desulfurizer cost. If necessary, this in-situ desulfurization method can combine with a fine desulfurization process to obtain a higher desulfurization rate economically.

Through Equations 3 and 4, for a certain required H₂S concentration, the additional quantity of in-situ desulfurizer can be calculated.

Application of Prediction Model in 5 L Fermentation

The prediction model was applied in 5 L fermenters. Firstly, the control fermentation without desulfurizer was performed, and the amount of H₂S was recorded. In this experiment, the H₂S concentrations in biogas were assumed to be demanded below 120, 200, and 100 ppmv when FeCl₂, FeCl₃, and Fe(OH)₃ were added as in-situ desulfurizers, respectively. Then \(m_r\) can be calculated, and \(x\) can be obtained through Equation 4. Dosage \(m\) was determined by Equation 3. Table 2 lists the calculated dosage of the three in-situ desulfurizers, as well as the actual desulfurization efficiency after adding them. Furthermore, the changes of biogas yield, CH₄ concentration, and pH are shown in Fig. 3; the changes of H₂S concentration are shown in Fig. 4.

Consistent with the results in bottles, adding desulfurizers did not promote or restrict biogas or methane production in 5 L fermentation, although the pH values were slightly lower than the control, especially in the fermenter with FeCl₃. In Fig. 3 (c), the pH decreased from 7.4 to 6.2 quickly in the first three or four days, corresponding the hydrolysis and acidogenesis stages with acid accumulated in anaerobic fermentation, and then it rose slowly to about

![Fig. 2. Correlation curve and equation of desulfurizer concentration in liquid and H₂S concentration in biogas.](image)

Table 2. Addition amounts of FeCl₂, FeCl₃, and Fe(OH)₃ under different desulfurization requirements, and actual desulfurization efficiency.

<table>
<thead>
<tr>
<th>In-situ desulfurizer</th>
<th>Required H₂S concentration (ppmv)</th>
<th>Dosage prediction</th>
<th>Experiment results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x) (mmol L⁻¹)</td>
<td>(m) (mmol)</td>
<td>(m) (mmol)</td>
</tr>
<tr>
<td>FeCl₂</td>
<td>(\leq 120)</td>
<td>4.31</td>
<td>2.50</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>(\leq 200)</td>
<td>3.86</td>
<td>1.60</td>
</tr>
<tr>
<td>Fe(OH)₃</td>
<td>(\leq 100)</td>
<td>1.99</td>
<td>1.68</td>
</tr>
</tbody>
</table>
7.2, demonstrating the subsequent methanogenesis stage. Fig. 4 indicates that the production of H\textsubscript{2}S also mainly occurred during hydrolysis and acidogenesis stages, and the H\textsubscript{2}S content of the control went up to 4918.4 ppmv on the fourth day of fermentation. In the fermenters adding \textit{in-situ} desulfurizers, the peak contents of H\textsubscript{2}S ranged from 200 to 600 ppmv. The average H\textsubscript{2}S concentrations of 5 L fermentation are given in Table 2. When FeCl\textsubscript{2} was used as desulfurizer, the actual H\textsubscript{2}S concentration was 163.0 ppmv, which was worse than required (120 ppmv), but still relatively close. When FeCl\textsubscript{3} and Fe(OH)\textsubscript{3} were applied, the H\textsubscript{2}S concentrations in biogas were 180.3 and 89.4 ppmv, respectively, which were close to the required desulfurization level (200 and 100 ppmv), and even a little better. From bottles to fermenters, the working volume increased by 140 times, and the prediction model showed good adaptability and effectiveness.

In the microscopic mechanism, a process of \textit{in-situ} desulfurization will include the competition and collaboration of microorganisms, reactions of sulfur and iron, etc. [6, 16]. But for the prediction of desulfurizer dosage, this simple and practical method can be applied regardless of the complex principles. The calculated dosage should be adjusted flexibly based on the real operation. And according to the actual situations, the experiments for dosage prediction can change the scale, reactor type, batch or continuous feed, etc.

In this study, two batches of CM were used, and the VS of CM2 was much lower than CM1 due to the high sand content. For the experiments of adding FeCl\textsubscript{2} and FeCl\textsubscript{3}, CM1 was fed in bottles, while CM2 was fed in 5 L fermenters. But the prediction formulas calculated through bottle experiments were applied well in 5 L fermentation, indicating that the prediction models were not sensitive to the property fluctuation of CM. In a follow-up study, the influence of the substrate change to the prediction model should be evaluated in detail, and the adaptability of this method to other substrates also needs to be tested.

**Conclusion**

How to accurately determine the dosage of added desulfurizer is a key question to \textit{in-situ} desulfurization. In this study, three iron compounds (FeCl\textsubscript{2}, FeCl\textsubscript{3}, and Fe(OH)\textsubscript{3}) were selected as good-performing \textit{in-situ} desulfurizers for CM fermentation, and used in the modeling experiments. For the establishment of the prediction model, regardless of the complex reactions, the dosage of desulfurizer was simply divided into two parts: one part for consumption of released H\textsubscript{2}S and the other part for guaranteeing a certain desulfurizing level. With this idea, the prediction formulas were fitted and applied successfully in a 5 L fermentation system. To our knowledge, it is the first time that the prediction method for an \textit{in-situ} desulfurizer dosage has been proposed. The method could be verified and improved through more experiments in laboratory, and practices in actual biogas plants.

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References


